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Research Article
Anti-convulsant Effects of *Bongardia chrysogonum* L. Tuber in the Pentylenetetrazole-induced Seizure Model

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Abstract

Background and Objective: The dried tuber of *Bongardia chrysogonum* (L.) is a popular folk remedy for its use in the treatment of epilepsy in traditional medicine. The study aimed to evaluate the anti-oxidant and anti-convulsant activity of *B. chrysogonum* ethanolic-aqueous extract using the Pentylenetetrazole (PTZ) kindling animal model. **Materials and Methods:** Male mice were randomly selected and divided into 9 experimental groups including: Control group, pentylenetetrazole kindled mice, positive mice group receiving valproate (200 mg kg^{-1} p.o.) a classic anticonvulsant drug and 3 groups receiving *B. chrysogonum* tuber-ethanolic or aqueous extract at a doses of (600, 900 and 1200 mg kg^{-1} p.o.). All groups, except the control, were kindled by 11 injections of PTZ (40 mg kg^{-1}, i.p.). All groups, except the control group, were tested at 12th PTZ challenge dose (75 mg kg^{-1} i.p.). The exhibited phases of seizure (0-6) were observed and noted; moreover, anti-oxidant effect of these extract was examined in *in vitro* study by using a spectrophotometric technique. The significance of differences between groups were determined using one-way analysis of variance (ANOVA) followed by post hoc test, Dunnett's multiple comparison tests. **Results:** The data showed that both valproate and *B. chrysogonum* tuber extracts delay the onset of convulsions, decrease duration of the seizure and reduced mortality significantly ($p<0.05$). In addition, *B. chrysogonum* showed a wide range of scavenging capacities for free radicals, which may underpin the effective *in vivo* seizure suppression. **Conclusion:** It was concluded that *B. chrysogonum* L. tuber extracts display anti-oxidant, free radical scavenging properties *in vitro* and, in mice, provides new scientific evidence for the anti-seizure properties of *B. chrysogonum.*

Key words: *Bongardia chrysogonum* L., tubers, epilepsy, kindling model, anti-oxidant, ethnomedicine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epilepsy is a common chronic neurological disorder, characterized by recurring seizures that affects all humans of all ages, races and ethnic backgrounds[1]. Different types of epilepsy have different causes depending on the part of the brain involved affecting approximately 70 million people and accounts for about 1% of the global burden of disease[2]. Major concerns in this disease are that, despite abundant availability of anti-epileptic drugs, seizures are not controlled in about 30% of the people with epilepsy and difficulties accessing adequate treatments. Furthermore, undesirable side-effects of the drugs used clinically often render treatments difficult so that the demand for new anti-epilepsy drugs exists[3]. The long-time seizure induced neuronal activity might result in neurological changes and finally result in neuronal death[4]. Oxidative stress and free radical production are of the most important mechanisms by which neurological disorders occur such as epileptic seizures[5]. The development of new, affordable and accessible pharmacological agents that can overcome treatment limitations has become a major goal in epilepsy research[6]. Natural products from folk remedies have contributed significantly to the discovery of modern drugs and are now considered as an alternative source for the discovery of anti-epileptic drugs with novel structures and better efficacy and safety profiles[7,8].

Bongardia chrysogonum L. is a perennial tuber bearing plant and a member of the Berberidaceae family[9]. It is a small plant native to the Eastern Mediterranean area[10] with a height of 30-50 cm. It has hairy and long leaves at the bottom and yellow flowers, commonly known in the Middle East "Urf El Deek". Preparations of the plant have been used in folk medicine to manage epilepsy, convulsions, pain and spasms for many years. Their effectiveness are widely acclaimed among rural communities of Jordan, Syria, Iran, Africa, Turkey and Afghanistan, where the tubers of this species are used in these regions in the form of 2-3% decoction to treat urinary tract infections, haemorrhoids and prostate hypertrophy[11,12] for epilepsy[13] and cancer cases[14] as well as diabetes[15]. The tubers of *B. chrysogonum* contain 1.76% fat, 2.76% glucose, 2.20% saccharose, 12% saponin and 0.11% alkaloid[16]. There is evidence implying anti-epilepsy effects of *B. chrysogonumin* on Jordanian traditional medicine; this study aimed to examine the anti-oxidant and anti-convulsant properties of the tuber extract in an experimental model of epilepsy in mice to establish a pharmacological basis for its anti-epileptic uses in folk medicine.

MATERIALS AND METHODS

Plant collection and authentication: Fresh tuber pieces of *B. chrysogonum* L. were collected from North West Amman, Jordan. The tubers were identified and authenticated by Professor Suleiman Al-Olimat, Department of Pharmaceutical Sciences; Faculty of pharmacy, The University of Jordan and a voucher specimen of the plant (*B. chrysogonum*-009) has been deposited in the herbarium of the University of Jordan for future reference.

Extraction: Fresh pieces of *B. chrysogonum* tuber were air-dried at room temperature. Tuber pieces of the plants were ground into fine powder and was extracted twice at the room temperature (25±1°C) for 72 h by the percolation method using 2 L (80%) ethanol or water with continuous shaking. The combined crude extracts were next filtered using 125 mm Whatman's filter paper No. 1 and then the solvents were evaporated to dryness under reduced pressure using rotary evaporator (HeidolphLaborota, Germany). The residues were further subjected to dryness by incubating them for 8 days at room temperature. The percentage yield was 15.61%, representing 78.05 g extraction from the 500 g of dried tubers. The crude extracts were either used directly or stored in an air-tight glass container at 5°C in a refrigerator for future use. For the experiments, 10% (w/v) *B. chrysogonum* crude extract were prepared.

Drugs and chemicals: All chemical and drugs were all purchased from (Sigma Chemical Co. USA). All solvents were of analytical grade.

In vitro anti-oxidant activity of *B. chrysogonum* extracts

Assay of direct free radical scavenging: Radical scavenging activity for both extracts was determined by a spectrophotometric method based on the reduction of a methanolic solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH)[17,18]. The percentage of scavenged DPPH calculated according the following formula[18]:

$$\% \text{ scavenged DPPH} = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (1)$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Tests were carried out in triplicate and ascorbic acid was employed as reference.

Scavenging capacity towards hydroxyl ion ($^{\cdot}$OH): The hydroxyl radical ($^{\cdot}$OH) scavenging activity of the plant extracts

were measured using the deoxyribose method[19]. BHT was used as positive control. The ·OH scavenging activity was calculated in accordance with the following formula[19]:

$$(\%)\ \text{scavenged}\ {}^{\cdot}OH = \frac{\text{Difference in absorbance of sample}}{\text{Difference in absorbance of blank}} \times 100 \quad (2)$$

Scavenging capacity towards hydrogen peroxide (H_2O_2): The H_2O_2 scavenging activity of extract was determined by hydrogen peroxide scavenging capacitymethod[20]. Percentage of hydrogen peroxide scavenging of both extracts was calculated as follows[20]:

$$\%\ \text{scavenged}[H_2O_2] = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (3)$$

where the A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Tests were carried out in triplicate and BHT was employed as reference.

Animals: Healthy young male albino Swiss mice, weighing 25-40 g, were used. The animals were kept and maintained under laboratory conditions (25°C), humidity and 12:12 h light-dark cycle at Al-Isra University animal house and were allowed free access to food (Standard pellet) and water ad libitum. The animals were acclimatized for at least 1 week before being used for experiments. All procedures concerning animals were carried out in accordance with Jordanian regulations for animal experimentation and care and were approved by the committee of institutional animal care and use (Protocol and Ethical approval memo number (IU/FP/120 dated 7th January 2014). The study commenced on the (21st March, 2014 and lasted for duration of 11 months). All experimentation were carried out in the Pharmacology Research Lab at the Faculty of Pharmacy at Isra University, Amman-Jordan. Adequate measures were taken to avoid any pain or discomfort to the animals during handling or experimentation.

Acute toxicity testing: Acute toxicity of ethanolic and aqueous extract of *B. chrysogonum* were carried out following the method described by the Organization for Economic Cooperation and Development OECD guideline No: 423 (OECD)[21]. All the animals were acclimatized to laboratory conditions for 2 weeks before commencement of experiment. The mice were left unfed for 12 h and divided into 7 groups of 5 each. Six groups were administered with graded doses of the extract (75, 250,500, 1000, 1500 and 3000 mg kg^{-1} p.o.). The control mice were given (10 mL kg^{-1}) of the vehicle (DW). All rats were then allowed free access to food and water and were observed for behavioral and physiological variation initially continuously for 4 h, followed by 4 hourly stints for 12 h and thereafter once daily for 14 days. The monitoring of the parameters commenced immediately after administrating the extract, for signs of toxicity, which included but were not limited to paw-licking, motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, sensations, pilo-erection, ptosis, lacrimation, exopthalmos, salivation, diarrhoea, writhing, skin colour, respiratory rate and mortality[22].

Experimental design: Male mice were randomly divided into 9 experimental groups each (n = 6). Pentylenetetrazole-induced convulsion: PTZ (40 mg kg^{-1} i.p.) was used to induce clonic-tonic convulsion in mice. Group 1 received vehicles (10 ml kg^{-1} $dose^{-1}$ Distilled Water orally (DW) , Group 2, 3 and 4 received ethanolic extract at different dose levels (600, 900 and 1200 mg kg^{-1} p.o.), Group 5, 6 and 7 received aqueous extract at different dose levels (600, 900 and 1200 mg kg^{-1} p.o.), group 8 was allotted for standard anti-convulsant drug (valproate 200 mg kg^{-1} orally) and group 9 control received (10 mL kg^{-1} $dose^{-1}$ distilled water without any treatment). All experiments were performed between 9.00 am and 2 pm in the laboratory of the department. The drug solutions were prepared freshly each time.

Kindling: All animals except the control group (group 9) were kindled by a total of 11 injections of PTZ (40 mg kg^{-1} i.p.). PTZ (Sigma) was dissolved in sterile isotonic saline. Each administration was carried out every 2nd day and for a period of 22 days. Mice were observed for 30 min after the last drug administration. After an additional 30 min, the mice were observed for lethality before returning to the home cage. The challenge dose of 75 mg kg^{-1} PTZ was injected to the kindled mice on 26th day (the test day), which could produce convulsions (tonic-clonic) and lethality. In the treatment groups (valproate and different doses of *B. chrysogonum*), PTZ was administrated 30 min after the 1st treatment with valproate and different doses of *B. chrysogonum*. Immediately after PTZ administration mice were observed for (1) Onset of convulsion, (2) Duration of convulsion. If no general seizure did occur during a 30 min period of observation, the animal was considered protected, the percentage survival was recorded for each group. However, the exhibited phases of seizure (0-6) were observed and categorized using the following scale for 30 min after the PTZ injection. The scale introduces 6 phases as follows; 0: No response, 1: Ear and facial twitching, 2: Convulsive waves axially through the body, 3: Myoclonic body jerks, 4: Generalized clonic convulsions turn

over into side position, 5: Generalized convulsions with tonic extension episode and status epilepticus, 6: Mortality[23]. Mice which experience lethal convulsions were excluded from the study. Mice that exhibit seizures with stage 4 or 5 are considered kindled and used in the study, the ability of plants extract to prevent seizure or delay/prolong the latency or onset of tonic clonic convulsion was considered to be an indication of anti-convulsant activity.

Statistical analysis: Results of the experiments and observations were expressed as Mean±Standard Error of Mean (SEM). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by post hoc test, Dunnett's multiple comparison tests[24]. A $p \leq 0.05$ level of significance was considered for each test. Unpaired t-test was used for analysing data obtained from the preliminary experiments. All the statistical analysis were performed using Graph Pad Prism version 6 software(Graphpad, La Jolla, CA, USA).

RESULTS AND DISCUSSION

***In vitro* antioxidant activity of *B. chrysogonum* tuber extracts:** Both extracts of *B. chrysogonum* successfully scavenged the free radical species studied. The DPPH free radical scavenging ability of both extracts increased together with the increase in concentration. Based upon the measured EC_{50} values, the DPPH quenching ability of *B. chrysogonum* water and ethanolic extracts and the standard ascorbic acid was found to be similar, 40±1.24, 32±1.10 and 27±0.04 μg mL^{-1}, respectively . The extracts also were capable of inhibiting $OH^{\cdot}$ radical formation in a concentration-dependent manner. The EC_{50} value of aqueous 180+0.89 μg mL^{-1} and ethanolic 140+1.40 μg mL^{-1} extracts were significantly ($p<0.05$) lower than that of BHT 16.44+0.04 μg mL^{-1}. Both extracts of *B. chrysogonum*, display a concentration-dependent scavenging of H_2O_2. Based on the EC_{50} values, the scavenging capacity of aqueous extract 110±1.25 μg mL^{-1} was significantly ($p<0.05$) higher than that of ethanolic extract 160+1.03 μg mL^{-1}. The EC_{50} value of BHT was found to be 65±0.2 μg mL^{-1}. The EC_{50} values indicated that both tested aqueous and ethanolic extract of *B. chrysogonum* roots display scavenging activity in a concentration-dependant manner, suggesting that these extracts possess a neuroprotective activity that may play a role in countering oxidative damage partly underpinning PTZ-induced seizure.

Acute toxicity study: Acute oral toxicity studies revealed the non-toxic nature of the *B. chrysogonum* tuber. Treatments with ethanolic extract and aqueous extracts of *B. chrysogonum* tuber did not show any major behavioural changes, sign and symptoms of toxicity and mortality up to 3000 mg kg^{-1} dose after 14 days of study. This indicated that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 3000 mg kg^{-1} body weight, the LD_{50} of B. *chrysogonum* tuber extract will be $\geq$3000 mg kg^{-1} and thus it is relatively safe and non-toxic to rats[25] in acute usage.

PTZ-induced kindling behavioural observations, dose response curve: PTZ kindling model was used to induce epileptic seizures in mice, to observe different seizure phases (0-6) in a preliminary experiment. Fifty male mice were randomly selected and divided into 5 experimental groups (n = 10). On the test day, mice in each group separately received single injections of PTZ as follows: Group 1, DW p.o., Group 2, 3,4 and 5 received 20, 40, 60,75 mg kg^{-1} i.p respectively as shown in (Fig. 1).

Effect of the ethanolic and aqueous extracts *B. chrysogonum* tuber on PTZ kindling in mice (40 mg kg^{-1} i.p.): Oral administration of stepwise, escalated doses of *B. chrysogonum* both ethanolic and aqueous extracts, the plant produced significant protective effect on the development of kindling using PTZ (40 mg kg^{-1} i.p.) when administered 30 min prior to PTZ. Extracts at doses (75 up to 1500 mg kg^{-1} p.o.) produced a significant increase ($p<0.05$) in protection against seizure compared to the control group as shown in Fig. 2.

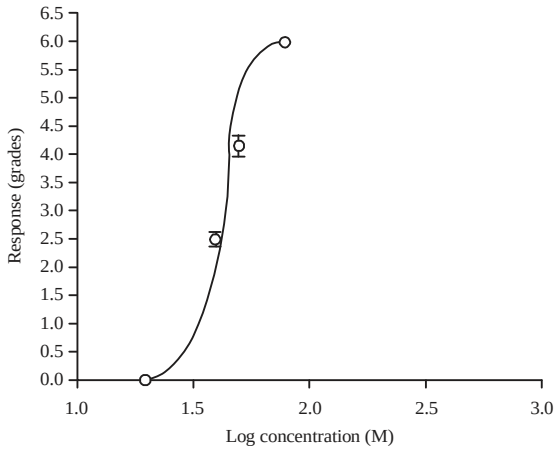

Fig. 1: Illustrate the PTZ-induced kindling dose response curve
Data are expressed as Mean±SEM

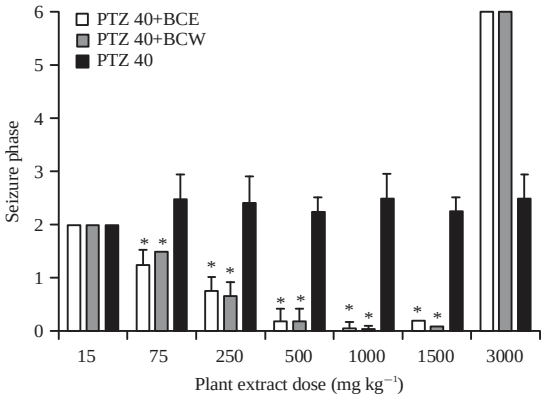

Fig. 2: Dose-response effect of *B. chrysogonum,* BCE: ethanol extract, BCW water extract after a dose of (40 mg kg^{-1} i.p.) of PTZ

Data are expressed as Mean±SEM. *indicates a significance difference where (p<0.05)using t-test

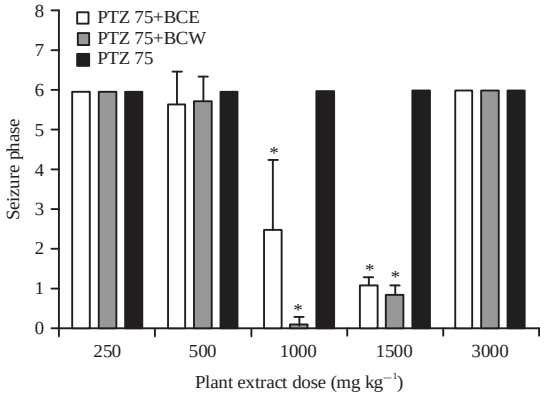

Fig. 3: Dose-response effect of *B. chrysogonum*, BCE: Ethanol extract and BCW water extract followed by a dose of (75 mg kg^{-1} i.p.) of PTZ

Data are expressed as Mean±SEM. *indicates a significance difference where (p<0.05)using t-test

Effect of the ethanolic and aqueous extracts *B. chrysogonum* tuber on PTZ-induced seizure and death (75 mg kg^{-1} i.p.): PTZ at 75 mg kg^{-1} i.p. induced seizure and death as shown in Fig. 3. Oral administration of stepwise, escalated doses of *B. chrysogonum* ethanolic and aqueous extract , where the extract of the plant at low doses did not significantly alter the onset of seizure whereas relatively high doses of the plant extract (1000-1500 mg kg^{-1} p.o.) produce significant protection of mice against PTZ-induced seizure (*: p<0.05).

Effect of the water extract of *B. chrysogonum* on PTZ-induced kindling: The *B. chrysogonum* water extract did not show toxicity (up to 1200 mg kg^{-1} p.o.) and demonstrated

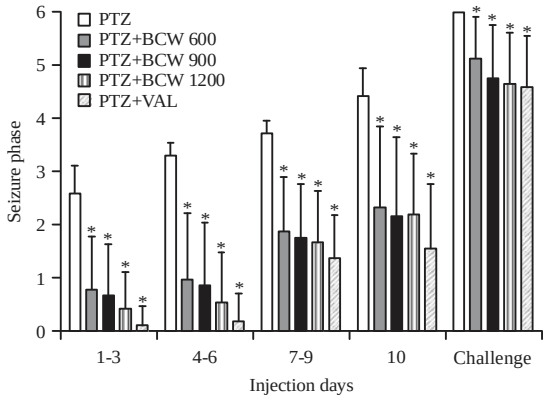

Fig. 4: Effect of *B. chrysogonum* water extract at 3 different doses (600, 900 and 1200 mg kg^{-1} p.o.) pretreatment on PTZ-induced kindling intensity

Data are expressed as Mean±SEM. The significance of differences between groups was determined using one-way analysis of variance (AVOVA) followed by Dunnett's multiple comparison test. *indicates a significance where (p<0.05). Experimental groups from 1-10 days used with PTZ (40 mg kg^{-1} i.p,), Experimental groups in challenge day used with a PTZ dose of 75 mg kg^{-1} i.p.)

a significant reduction in seizure phase of mice treated with a different doses of water extract p.o., as compared with mice group given PTZ only (Group 1) as shown in Fig. 4, (p<0.05) using one-way ANOVA. The *B. chrysogonum* aqueous extract produced dose-dependent significant protection of the mice against PTZ-induced seizures. The delay in seizure onset and antagonized seizure responses were comparable with the reference anti-convulsant, valproic acid.

Effect of the ethanolic extract of *B. chrysogonum* tuber on PTZ-induced kindling: The plant ethanolic extract did not show toxicity (up to 1200 mg kg^{-1} p.o) and demonstrated a significant reduction in seizure phase of mice treated with tuber extract, as compared with mice group given PTZ only (Group 1) (p<0.05) using one way ANOVA Fig. 5. *B. chrysogonum* ethanolic extract produced dose-dependent significant protection of the mice against PTZ-induced seizure. The delay in seizure onset and antagonized seizures were comparable with the reference anticonvulsant valproic acid. Both *B. chrysogonum* extracts strongly protected mice against convulsions (with a similar concentration profile). When compared to valproate, of *B. chrysogonum* at a dose of 1200 mg kg^{-1} provided robust protection against PTZ-induced seizures by nearly 66 and 61% for water extract and ethanolic extract respectively, as compared with 72% for valproate. The percentage of protection of both extracts on the survival and death of the mice in comparison with standard anti-convulsant and valproate are shown in Table 1.

Table 1: Effect of ethanolic and aqueous extract of *B. chrysogonum* tuber on PTZ kindling in mice

Group numbers	Onset of convulsion (Sec)*	Dead mice/total mice	Protection (%)	Mortality (%)
Group 1: Distilled water (D.W.), at a dose of (10 mL kg^{-1} p.o.) followed by (75 mg kg^{-1} IP) PTZ	91±14	18/18	0.00	100.00
Group 2: Ethanolic extract (600 mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p.	120±16	8/18	55.56	44.40
Group 3: Ethanolic extract (900 mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p	160±16	8/18	55.56	44.40
Group 4: Ethanolic extract (1200mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p.	180±18	7/18	61.11	38.88
Group 5: Aqueous extract (600 mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p.	168±24	8/18	55.56	44.40
Group 6: Aqueous extract (900 mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p.	203± 39	7/18	61.11	38.88
Group 7: Aqueous extract (1200 mg kg^{-1} p.o.), followed by PTZ 75 mg kg^{-1} i.p.	305±22	6/18	66.66	33.33
Group 8: VAL (200mg kg^{-1} p.o.) prepared in D.W. Followed PTZ 40mg kg^{-1} i.p.	A	5/18	72.22	27.77
Group 9: D.W. (10 mL kg^{-1})	A	0/18	100.00	0.00

A = absence of convulsion, *values are mean with SD in parenthesis (n = 18). Total number of mice (n = 18) for each group, D.W: Distilled water, VAL: Valproic acid, PTZ: Pentylenetetrazole

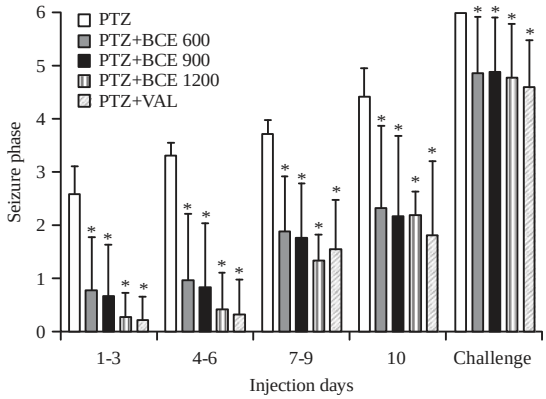

Fig. 5: Effect of *B. chrysogonum* ethanol extract at 3 different doses (600, 900 and 1200 mg kg^{-1} p.o.) pretreatment on PTZ-induced kindling intensity

Data are expressed as Mean±SEM. The significance of differences between groups was determined using one-way analysis of variance (AVOVA) followed by Dunnett's multiple comparison test. *indicates a significance where ($p<0.05$). Experimental groups from 1-10 days used with PTZ (40 mg kg^{-1} i.p,), Experimental groups in challenge day used with a PTZ dose of 75 mg kg^{-1} i.p.)

Botanicals are increasingly used by people with epilepsy worldwide. However, despite abundant data on the anti-convulsant properties of many herbal remedies in complementary and alternative medicine, there are very few scientific studies assessing efficacy and safety of these plants in animal models. In this study, efforts were made to investigate the anti-convulsant potential of *B. chrysogonum* root extracts against pentylenetetrazole-induced seizures in mice and antioxidant properties on free radical scavenging *in vitro*. Results of this animal study indicated that *B. chrysogonum* ethanolic and aqueous extracts possesses a dose-dependent and significant ($p<0.05$) anti-convulsant activity in mice; both extracts increased latency to onset and decreased duration of clonic convulsion in pentylenetetrazole model as compared with control group. The *B. chrysogonum* extracts also successfully scavenged the range of reactive species studied. The analysis of values indicated that both tested ethanolic and aqueous extracts of *B. chrysogonum* root display scavenging activity in a concentration-dependent manner and via this potent anti-oxidant effect could act as neuroprotective agent attenuating the oxidative stress induced by PTZ kindling.

Determination of median lethal dose value of plants used by traditional medicine practitioners using an acute toxicity study is important because it provides information regarding the margin of safety of the plant[21,22]. The relatively high LD_{50} value (3000±50 mg kg^{-1} p.o.), obtained in this study for both extracts suggests that the plant extract is safe and non-toxic

in mice. To the best of our knowledge, this is the first report on the anti-convulsant and antioxidant effects of *B. chrysogonum* in the literature and provides evidence and support for its use as a natural supplementary remedy in the management of epileptic seizures.

It was earlier reported by Tanker[16] that the tubers of *B. chrysogonum* contains 12% saponin and 0.11% alkaloids. Phytochemical analysis of the plant tuber for different classes of secondary metabolites also revealed the presence of 4 types alkaloids of that were lamprolobine, bonzakaline, lupanine and palmatrubine[26-28]. The pharmacological potential of the plant could be associated with the presence of another class of secondary metabolites, namely the triterpenoid saponins. Seven types were isolated; these were leontoside A, leontoside D, hederacoside A, symphytoxide B and hederagenin alpha-L-arabinopyranosyl, hederagenin, beta-D-glucopyranosyl and hederagenin beta-D-glucopyranosyl ester[29,30]. These secondary metabolites could account for the observed protective effects of the plant.

Our present state of knowledge of the chemical constituents of *B. chrysogonum* is limited. It is difficult to identify the constituents in the tuber responsible for anticonvulsant activity. Because the tuber extracts major constituents as saponin and alkaloids, one of these chemical compounds are speculated to account for the observed anti-convulsant effect. But any other possible constituents present in the plant tuber apart from saponin and alkaloids may account for the observed anticonvulsant activity and this needs further investigation. However, both saponins and alkaloids, the major chemical components of *B. chrysogonum* tuber have been reported in the literature to exhibit anti-convulsant activity in experimental seizure models, such as PTZ by various authors[30-35]. Testing isolated pure compounds for the anti-convulsant activity will be interesting.

The mechanism of action of most anti-convulsant drugs have been broadly divided into 3 major categories: 1st, promoting or prolonging the inactivated voltage activated Na^+ channel, 2nd, enhancing and facilitating Gamma Amino Butyric Acid (GABA) mediated synaptic transmission. Third category act by reducing or limiting the flow of Ca^{2+} channels through T-type voltage activated channels[36]. Authors used an experimental model of seizures with PTZ, a selective blocker of the chloride channel coupled with the GABA receptor complex and considered the gold standard for screening potential anti-convulsant compounds. Pentylenetetrazole administration parenterally has consistent convulsant actions in mice. PTZ initially produces myoclonic jerks which subsequently become generalized and may lead to a generalized tonic-clonic seizures[37]. The plant tuber extracts were effective in PTZ-induced convulsion model; the average onset duration and intensity of convulsion were significantly reduced. Valporic acid is a known conventional anti-epileptic agent that increases turnover of GABA and thereby potentiates GABA ergic functions in some specific brain regions thought to be involved in the control of seizure generation and propagation. As expected, valporic acid pre-treated mice did not elicit convulsive episodes or show any mortality when treated with PTZ. Since the extract showed anti-convulsant effect against PTZ-induced seizure, it is possible that they may be interfering with GABA transmission to exert their anti-convulsant effects; this needs to be confirmed in follow-up studies and additional experiments to develop the exact underlying mechanism of anticonvulsant action of possible constituents of the plant after isolation of bioactives.

Recent studies[38] supported a major role of oxidative stress in the development of epilepsy; moreover classical experimental models of seizure (PTZ, Strychnine and picrotoxin) induce seizures via different mechanisms but share a common oxidative stress pathway, defined as imbalance in the levels of Reactive Oxygen Species (ROS) producing free radicals responsible for producing neuronal stress; oxygen which is necessary for many aerobic cellular reactions may undergo electron transfer reactions which generates highly reactive oxygen and free radicals such as hydroxyl or hydrogen peroxide radical which result in oxidative damage in the brain. Various defense systems exist in the brain to scavenge ROS, including glutathione, vitamin E, C and A. In this study, it was demonstrated that not only water but also ethanol extracts from *B. chrysogonum* tuber contain free radical scavengers that are modestly more concentrated in the water extract of the tubers. Obtained results show that the *B. chrysogonum* tuber extracts display a good scavenging capacity when compared with the reference compounds suggesting that these possess a neuroprotective activity that might be a key for ameliorating PTZ-induced seizures due to anti-oxidant activity.

Future work needs to be focused on testing *B. chrysogonum* plant extract in other different *in vivo* experimental models of epilepsy in mice[37] to acertain the activity and mechanism in anti-convulsion. In addition since the experimental epilepsy is mediated by oxidative stress and free radicals and it could be suggested that *B. chrysogonum* is able to prevent seizures by an antioxidant mechanism. Effects of *B. chrysogonum* extracts *in vivo* on the brain level of biochemical indices of oxidative stress in tissues in the kindled and non-kindled groups needs to be studied by measuring lipid peroxidation level, nitrite content, glutathione

reduced (GSH) concentration, superoxide dismutase and catalase activities in the mouse brain[38]. Finally, the plant tubers used in the study were based on traditional uses[13]; for comparison purposes it would be interesting to test other parts of the plant (leaf/flower/seed/fruit extracts).

CONCLUSION AND FUTURE RECOMMENDATION

This study illustrates for the first time that *B. chrysogonum* L. root extracts display anti-oxidant, free radical scavenging properties *in vitro* and, in mice, possess very effective anti-convulsant activity. These findings provided scientific claim to the usefulness of this traditional plant in neurological disorders like epilepsy. Acceptable efficacy and lack of acute toxicity suggest further studies for isolation of the neuroactive components and elucidation of the mechanism underlying anti-convulsant action.

SIGNIFICANCE STATEMENTS

This study discovered the anti-convulsant properties of *Bongardia chrysogonum* L. tuber extracts in the PTZ induced seizure model that can be beneficial for a novel epilepsy treatment. This suggests a radical and GABA-mediated mechanism. This study lends pharmacological credence to the folkloric ethnomedical use of the plant tubers as a natural supplementary remedy in the managements and control of epilepsy.

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